

In re Application of:  
Short and Keller  
Application No.: 08/876,276  
Filed: June 16, 1997  
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**In the claims**

Please cancel claim 42, without prejudice.

Please amend claim 19 follows:

19. (Currently Amended) A method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

- a) contacting a bioactive substrate that is fluorescent in the presence of the bioactivity or biomolecule of interest with a library containing a plurality of clones containing naturally occurring DNA from at least more than one organism;
- b) screening the library with a fluorescent analyzer that detects bioactive fluorescence, and
- c) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of naturally occurring DNA that encodes a bioactivity or biomolecule of interest.

20. (Previously added) The method of claim 19, further comprising obtaining DNA from a clone that is positive for an enzymatic activity of interest.

21. (Previously added) The method of claim 20, wherein the enzymatic activity of interest is from an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

22. (Previously added) The method of claim 19, wherein the library is generated in a prokaryotic cell.

23. (Previously added) The method of claim 1, wherein the library contains at least about  $2 \times 10^6$  clones.

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24. (Previously added) The method of claim 1, wherein the prokaryotic cell is gram negative.
25. (Previously added) The method of claim 19, wherein the clones are encapsulated in a gel microdrop.
26. (Previously added) The method of claim 19, wherein the analyzer screens up to about 15 million clones per hour.
27. (Previously added) The method of claim 19, wherein the clones are extremophiles.
28. (Previously added) The method of claim 27, wherein the extremophiles are thermophiles.
29. (Previously added) The method of claim 27, wherein the extremophiles are hyperthermophiles, psychrophiles, halophiles, psychrotrops, alkalophiles, or acidophiles.
30. (Previously amended) The method of claim 19, wherein the bioactive substrate comprises staining reagent C12-fluorescein-di-D-galactopyranoside (C12FDG).
31. (Previously added) The method of claim 19, wherein the bioactive substrate comprises a lipophilic tail.
32. (Previously added) The method of claim 19, wherein the clones and substrates are heated to enhance contacting of the substrate with the clones.
33. (Previously added) The method of claim 32, wherein the heating is to a temperature of about 70°C.

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34. (Previously added) The method of claim 32, wherein the heating is for about 30 minutes.
35. (Previously added) The method of claim 19, wherein the fluorescent analyzer comprises a fluorescence activated cell sorting (FACS) apparatus.
36. (Previously added) The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA is stable at a temperature of at least about 60°C.
37. (Previously added) The method of claim 19, wherein the library is an expression library.
38. (Previously added) The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA possesses enhanced enzymatic activity of interest compared to that of a wild-type enzyme.
39. (Previously added) The method of claim 19, wherein the method further comprises biopanning the expression library prior to contacting with the substrate.
40. (Previously added) The method of claim 19 further comprising obtaining DNA from a clone identified in step c) that is positive for an enzymatic activity of interest and comparing the enzymatic activity of a DNA expression product from the clone with that obtained from such a clone into whose DNA at least one nucleotide mutation has been introduced, wherein a difference in enzymatic activity is indicative of the effect upon the enzymatic activity of interest caused by introduction of the at least one nucleotide mutation.
41. (Cancelled).

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42. (Previously added) The method of claim 19, wherein each clone contains DNA obtained from a single organism.
43. (Previously added) The method of claim 19, wherein the library is a multispecies library.
44. (Previously added) The method of claim 43, wherein the library is generated from a mixed population of uncultured organisms.
45. (Previously added) The method of claim 43, wherein the library is generated from isolates.
46. (Previously added) The method of claim 40, wherein the mutation is introduced by error-prone PCR, oligonucleotide directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis and exponential ensemble mutagenesis.